

Effect of humic acid application methods on yield and quality of Mungbean (*Vigna radiate* L.)

Muhammad Javed¹, Muhammad Tahir¹, Asif Iqbal¹, Muhammad Abdullah Saleem^{1*}, Muhammad Tahir Naveed¹, Misbah Kausar²

¹Department of Agronomy, University of Agriculture Faisalabad, Faisalabad 38000, Pakistan.

² Department of Botany, University of Punjab, Pakistan

*Corresponding author: abdullahsaleem65@gmail.com

ABSTRACT

The application methods of nutrients play a pivotal role in enhancing crop production. Soil amendment aids in nutrient fixation, while foliar application may improve penetration through the cuticle, thereby reducing nutrient losses. An experiment conducted at the University of Agriculture Faisalabad (Pakistan) from March to June 2017 aimed to evaluate different application methods for humic acid in mung beans using a Randomized Complete Block Design. The treatments included were [H1= Control (dry seed), H2= hydro priming, H3=seed priming with 2% humic acid H4=4% humic acid, H5= 2 kg humic acid ha⁻¹, H6= 4 kg humic acid ha⁻¹, H7=0.1% spray of humic acid and H8=0.2% spray of humic acid. Results showed that no. of pod bearing branches (7.33), no. of pods per plant (18.00), no. of seeds per pod (9.66), 1000-seed weight (44.00 g), plant height (45.16 cm), seed yield (1487.70 kg ha⁻¹), biological yield (5191.30 kg ha⁻¹), harvest index (28.64 %) and protein contents (25.20 %) were statistically significant in the treatment H6 (4 kg humic acid ha⁻¹). The lowest values of all these parameters were obtained from treatment H1 in which no treatment was applied. In conclusion, soil application of humic acid, especially at a rate of 4 kg ha⁻¹, significantly increased mung bean yield and quality. The findings suggest that this dose was particularly effective, and future research could explore higher doses to optimize humic acid levels for further enhancing mung bean production.

Keywords: Humic acid, mungbean, seed priming, foliar application, soil application, yield.

INTRODUCTION

Agriculture poses a significant impact on global economy and provides the mankind with food, shelter and energy requirements including milk, and meat from livestock in addition to grains and pulses from crop sources [1]. Poverty alleviation and zero hunger in the global scenario are directly linked to the provision of healthy food to individuals [2]. One of the most essential components of the food stuff of the human being is protein. Legumes are essential in cropping systems because of their capacity to form symbiotic associations with Rhizobium, leading to nodule formation and nitrogen fixation [3-5].

This process converts atmospheric nitrogen into nitrates and nitrites, making it readily available for plant uptake. Legume crops contribute to increased soil productivity and nutrient efficiency [6, 7]. They have a significant and sustainable impact on global agriculture, with an annual production of 175 million tonnes along with nitrogen fixation [8]. Mung bean, being a leguminous crop, has the advantage of thriving in soil having low nitrogen contents [9, 10]. Pulses under legumes are the most important sources to provide the non-animal protein. Pulses are vital edible crops and the most economical protein source. Green gram, also known as mung bean, is cultivated in tropical and subtropical climates and is a crucial legume in terms of food production. Mungbean is an excellent source of lysine, although it lacks a significant amount of methionine [11, 12]. Mungbean is a very imperative legume crop in Asia and has been grown since ancient times. Mungbean is a great source of minerals for pregnant ladies, that is Zn (3 mg), Fe (7.3 mg) and Ca (124 mg), foliate (549 mg) per hundred gram of seed [13]. It consists of approximately 24% protein out of which globulin shows 60% while albumin contributes 25% [14]. Its anti-inflammatory properties can be attributed to

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significant levels of proteins, polysaccharides, polyphenols, and its involvement in lipid regulating metabolism [15]. It also provides a healthy environment to the soil by fixing atmospheric nitrogen into the rhizosphere.

Out of six million hectares globally, the major cultivated area under the mungbean is in Asia. Mung bean cultivation is predominantly practiced in Asia, Australia, and Africa. Pakistan, Thailand, China, and India are the primary producers of mung bean in Asia [16, 17]. Presently, mung beans are grown globally, occupying approximately six million hectares of land annually, with Asia contributes 90% of total area [18, 19]. The average yield of mung bean is around 400 kg/ha, which is considered too low form potential [20]. Due to its significant nutritional value, the demand for mung bean is steadily increasing and mung bean exhibits a high potential to address food security concerns [21, 22].

In Pakistan, mung bean holds significant importance as one of the key kharif crops, cultivated twice a year. It is classified as a warm-season crop and is commonly grown after wheat crop. Mung bean is known for its ability to thrive in harsh environments and requires significantly less water than other crops [23-25]. However, the average yield of green gram in Pakistan is comparatively low [26]. Consequently, a substantial quantity of mung beans needs to be imported from other countries to meet domestic demand for pulses. In Pakistan, mung bean is cultivated on area of 0.14 million hectares, with production of 0.1188 million tons [27].

Similar to all living beings, crops require nutrients for growth and development [28]. All the nutrients are essential for plant structure, chemical reactions of photosynthates and the production of organic compounds [29]. Plants acquire both macro and micronutrients from organic and inorganic resources throughout the world [30, 31]. Chemical fertilization of the soil has led to decreased soil productivity over time. Many of the chemical amendments are causing deterioration of soil organic matter, humic substances and microbial activities due to their complexes in soil profile [32]. Alternatively, soil amendment with organic manures can be an easy and sustainable way to improve soil health and productivity for a longer period of time [33]. Organic materials are considered to increase soil physical and chemical characteristics, soil fertility, and carbon sequestration. Humic substances are also used for good crop production and soil health. Humic matter increases the uptake of minerals by stimulating the activity of microbes. Humic compounds have indirect effects on the soil chemical, biological and physical condition improvement [34]. Humic acid substances have been evaluated as soil improver when combined with

beneficial bacteria [35]. Humic acid solutions has initiated the uplifted the microbial activity by providing the bacterial residential media and environment [36], increased the nutrient uptake by pant roots [37]. Humic acid surged the plant growth and improved the soil health along with a positive impact on nutrient availability [38]. Fertilizer application methods are also key factors to consider for higher crop production as soil application promotes nutrient fixation and foliar application increases the chances of nutrient availability by direct penetration through cuticle [39]. Foliar applications increase the utilization of nutrients and reduce the environmental pollution by decreasing the fertilizers amount added to soil. Foliar application of one nutrient may increase the uptake of other ones from soil solution by increasing root growth [40]. Humic acid absorption efficiency through immature leaves by stomata was high and on leaf surface no accumulation was observed. Therefore, no burning and scorching of leaf noted [41]. Foliar use of humic mater and bio-stimulator has positive impact on fruits set and improve the cucumber yield [42]. The humic acid applied as foliar had helpful effect on plant total yield, fruit firmness, fruit no. and chlorophyll substance. Usually, foliar used of humic acid function as enhanced the qualitative and quantitative properties of the strawberry [43]. Seed priming helps in achieving high-quality crop establishment, increased Biological N-Fixation of pulses and gain extra advantages from the soil of less fertility. Seed priming is effective for legumes as it increased the yield of legumes considerably [44]. The seed priming of wheat with humic acid increased the germination process, percent seed germination and formed more root elongation. Seed priming with humic acid positively enhanced the germination of seeds and effective for root growth and shoots length [45].

To relish the benefit from the fact that humic acid provides vast assurance for agricultural use, , it was hypothesized that addition of humic acid through any method will increase plant growth and crop yield. Therefore, a field study was planned to assess different strategies of humic acid to enhance the yield and quality of mungbean as this crop is usually grown on neglected soils in Pakistan and the area under cultivation has decreased drastically in the recent decade.

Materials and Methods

Research Design and Location

Field study was carried out at University of Agriculture Faisalabad (Pakistan) (31° 26' 2.18" N, 73° 3' 53.6" E) using Randomized Complete Block Design (RCBD) having 3 replications.

Treatment Plant

The treatments included were:

H ₁ = Control (dry seed)
H ₂ = hydro priming
H ₃ =seed priming with 2% humic acid
H ₄ =4% humic acid
H ₅ = 2 kg humic acid ha ⁻¹
H ₆ = 4 kg humic acid ha ⁻¹
H ₇ =0.1% spray of humic acid
H ₈ =0.2% spray of humic acid

Characteristics of experimental soil:

Component	Value
Silt	39%
Clay	15%
Sand	46%
Saturation	32%
Electrical conductivity	2.0 dSm ⁻¹
Soil pH	7.69
Organic matter	0.57%
Nitrogen	0.042%
Potassium	182.9 ppm
Phosphorus	6.68 ppm

Cultivation

Mungbean (NIAB-MUNG 2011) was sown as a test crop in spring season of 2017 using seed rate 30 kg ha⁻¹. Soil applied treatments were amended at the time of sowing, seed priming of the required seeds was done 12 hours before sowing, whereas foliar sprays were done at 5-6 leaves stage. Recommended N:P:K (25:60:30 kg ha⁻¹) was applied through DAP, and SOP as nutrient sources.

Cultural Practices

Four irrigations, 21 DAS (Days after sowing), flowering stage, pod formation stage and grain development stage) was applied. Lectofin (50ml ha⁻¹) + Quizalofop-methyl (610ml ha⁻¹) was used to control seasonal broad-leaved weeds during the growth season. Crop was harvested on ripening after 90 DAS.

Data Recording and Analysis

At harvesting, 10 plants from each treatment were selected randomly and measured the parameters by following standard procedures. No. of pods plant⁻¹ and no. of seeds pod⁻¹ were counted manually. Plant height (cm) was measured by measuring tape. Electronic weighing balance was utilized for 1000-seed weight (g), seed yield (kg ha⁻¹) and biological yield (kg ha⁻¹). Seed protein contents (%) were measured by following Gaines [46] method. Harvest index (%) was calculated using formula $H.I = (\text{Grain yield}) / (\text{Biological yield}) \times 100$. [47] All the recorded data was analyzed using LSD test at 5% probability level [48] using Statistix software (V 8.1).

RESULTS AND DISCUSSION**No. of Pod Bearing Branches Plant⁻¹**

The data concerning pods bearing branches given in the table: 1 indicated that the humic acid application methods had significant effect on pods bearing branches. The statistically highest no. of pods bearing branches (7.33) were obtained where humic acid (4 kg ha⁻¹) was added to the soil (H₆). However, treatments H₅ and H₈ were statistically at par with treatment H₆. The lowest no. of pods bearing branches (5.00) was noticed from treatment H₁ where no humic acid was applied. Increased pod bearing branches were witnessed because of humic acid application. Humic acid played a role in hormonal activity of plants which regulates the endogenous hormonal mechanism. Positive response to uptake of soil nutrients by addition of humic acid exhibit significant increased in branches. Humic acid has positive effect to increasing no. of branches which are also documented by [49]. [50] also concluded that humic acid play role in increasing no. of branches. Humic acid also improves the primary and secondary metabolism of plants by inducing certain necessary changes [51].

No. of Pods Plant⁻¹

It is an important parameter for yield contribution. The data regarding no. of pods per plant are given in the (Table: 1) showed that the application of humic acid in all three methods significantly effect on no. of pods. Statistically more no. of pods per plant (18.00) were collected where humic acid @ 4 kg ha⁻¹ was added in soil (H₆) however, it was at par with the treatments: where humic acid @ 2 kg ha⁻¹ was added in soil (H₅) and foliar spray with 0.2 % humic acid solution (H₈) was applied. The lowest no. of pods per plant (13.00) was obtained from control. Humic acid has vital role to enhance soil aeration, water holding capacity and accelerate microbial activity. These components ensure the establishment optimum plant population which has direct influence on economic yield. Humic acid help in increasing chlorophyll content of plants which have key role in photosynthesis that increase carbohydrates accumulation of plants that ultimately influence the plant fruiting. Humic acid might influence plant hormones, such as auxins and cytokinins, which play crucial roles in regulating growth and development [52]. Humic acid may influence the activity of enzymes involved in various metabolic processes. This can affect

hormonal balance within the plant, potentially promoting flowering and pod setting. Humic acid have also been involved in accelerating ATPases which increase the cell enlargement, cell growth, increasing nutrient uptake and ion uptake [53].

No. of Seeds Pod-1

The data introduced in the table: 1 regarding no. of seeds per pod indicated that the humic acid application methods had significant impact on no. of seed pod-1. The statistically maximum no. of seed pod-1 (9.66) was observed where humic acid @ 4 kg ha⁻¹ was added in soil (H6) however, it was at par with the treatments: where humic acid @ 2 kg ha⁻¹ was added in soil (H5), foliar spray with 0.2 % humic acid solution (H8) was applied and foliar spray with 0.1 % humic acid solution (H7) was applied. The lowest no. of seed pod-1 (7.00) was obtained from the control (H1). Humic acid application positively enhanced the no. of seed per pod for the reason that it has important role to enhance chlorophyll contents in plants. More chlorophyll contents support photosynthesis process which enhances the more photosynthates it ultimately enhances the accumulation in the sinks [54]. Humic acid application increasing photosynthetic efficiency and promotes the seed development which increases the seed number in single pod [55]. Vanitha and Mohandass [56] also found higher number of seeds per pod with humic acid application. Soil application of humic acid might have played role in improving soil conditions and nutrient regulation ultimately enhancing the plant growth and development.

1000 Seed Weight (g)

The data analysis presented in Table 1 reveals a statistically significant increase in the 1000-seed weight. The highest recorded 1000-seed weight (44.00 g) was observed in the treatment where humic acid was applied at a rate of 4 kg ha⁻¹ in the soil (H6). Notably, this result was on par with the treatment employing humic acid at a rate of 2 kg ha⁻¹ (H5). In contrast, the control group (H1) exhibited the lowest 1000-seed weight (39.00 g). The introduction of humic acid into the soil positively impacted root growth and development, consequently enhancing nutrient uptake by the plants. This aligns with findings by Sharif, Khattak [57], who reported that humic acid, containing Phosphorus (0.2-1%), Carbon (51-57%), and Nitrogen (4-6%), can significantly augment crop yields by providing essential Nitrogen and

Phosphorus to plants. The beneficial effects of humic acid are attributed to its chelating capacity for nutrients and its influence on various soil properties, encompassing biological, chemical, and physical aspects. Additionally, the improved uptake of micronutrients facilitated by humic acid has a notable impact on the observed increase in 1000-seed weight [56].

Height of Plant at Maturity (cm)

The data presented in Table 1, focusing on plant height, underscores the significant impact of various humic acid application methods on enhancing plant height. The treatment with the highest statistically recorded plant height (45.167 cm) involved the addition of humic acid at a rate of 4 kg ha⁻¹ to the soil (H6). Notably, this result was comparable to treatments where humic acid was applied at 2 kg ha⁻¹ in the soil (H5), foliar spray with a 0.2% humic acid solution was administered (H8), foliar spray with a 0.1% humic acid solution was applied (H7), and seed priming was conducted with a 4% humic acid solution (H4). In contrast, the lowest plant height (36.00 cm) was observed in the control treatment (H1).

The utilization of humic acid emerges as a noteworthy factor in promoting plant growth. These findings align with the outcomes of previous research conducted by Memon, Bangulzai [50], further supporting the positive correlation between humic acid application and enhanced plant height as observed in the present study.

Seed Yield (kg ha-1)

The data in Table 1 reveals a significant enhancement in seed yield due to various humic acid application methods. The treatment with the highest statistically recorded seed yield (1487.70 kg ha⁻¹) involved the addition of humic acid at a rate of 4 kg ha⁻¹ to the soil (H6). Importantly, this result was comparable to the treatment where humic acid was applied at 2 kg ha⁻¹ (H5). Conversely, the control treatment (H1) exhibited the lowest seed yield at 761.00 kg ha⁻¹.

The increased seed yield attributed to humic acid application can be attributed to its positive effects on soil structure and crop productivity. Humic acid plays a crucial role in enhancing the absorption of mineral elements such as phosphorus, magnesium, nitrogen, potassium, and calcium. Phosphorus, in particular, is vital for tissue growth, cell division, root proliferation, and nutrient uptake, ultimately influencing economic yield. Humic acid has been

associated with increased plant tolerance to various environmental stresses, such as drought, salinity, and temperature extremes. Plants that are better able to withstand stress conditions are more likely to produce higher yields [58, 59].

Furthermore, humic acid contributes to increased grain yield and quality by improving soil fertility. Its effects on plant growth, whether direct or indirect, include influencing the plant cell membrane, enhancing mineral transport, promoting protein synthesis, acting as a plant hormone, impacting enzyme activity, and increasing photosynthesis. The solubilization of macro and microelements, along with the enhancement of the microbial community, further contributes to its positive influence on plant growth. These findings align with the results of previous research by Seyedbagheri [60] and Khan, Rashid [61]. According to Erro, Urrutia [62] humic acid has been found increasing the available phosphorus contents in the soil which are very helpful in root system development and grain production.

Biological yield (kg ha⁻¹)

Data showed in the table: 1 indicated that statistically highest biological yield (5191.3 kg ha⁻¹) was recorded where the soil application @ 4 kg ha⁻¹ humic acid was done (H6) however, it was at par with the treatment where humic acid @ 2 kg ha⁻¹ was added in soil (H5). The least biological yield (3990.0) was noted in control (H1). The reason behind increasing the biological yield is the use of humic acid that can enhance the rate of photosynthesis and respiration by improving the functioning of chloroplast and mitochondria. It stimulates growth of crops and enhanced the yield of the crops by affecting the different mechanisms involved in: protein synthesis, photosynthesis, cell respiration, enzyme activities, nutrient uptake and water uptake [54]. Use of humic acid increased the photosynthesis process, plant root respiration and chlorophyll density has resulted in better crop growth [63]. Humic acid application had significantly affected the biological yield, concluded by Iqbal, Anwar [64].

Table 1. Effect of humic acid application methods on yield and quality of Mungbean

Treatments	No. of pod bearing branches	No. of pods plant ⁻¹	No. of seeds per pod	1000-seed weight (g)	Plant height (cm)	Seed yield (kg ha ⁻¹)	Biological yield (kg ha ⁻¹)	Harvest index (%)	Seed protein content (%)
H ₁	5.00 d	13.00	7.00	39.00f	36.00 d	761.0 f	3990.0 f	19.05 e	21.00 f
H ₂	6.00 c	14.00 de	7.66 de	39.67 ef	37.77 cd	891.7e	4278.7 e	20.836 e	21.40 ef
H ₃	6.00 c	15.00 cd	8.00 c-e	40.00d-f	40.40 bc	1009.7de	4382.0 de	23.021 d	22.10 de
H ₄	6.33 bc	15.00 cd	8.33 b-d	41.00c-e	42.07 a-c	1105.0 cd	4523.0 cd	24.41cd	22.30 cd
H ₅	7.00 ab	17.00 ab	9.33 ab	43.00ab	43.30 ab	1396.7a	5067.0 a	27.56 ab	24.90 ab
H ₆	7.33 a	18.00 a	9.66 a	44.00 a	45.17a	1487.7 a	5191.3 a	28.65 a	25.20 a
H ₇	6.33 bc	16.00 bc	9.00 a-c	41.33cd	42.07 a-c	1187.3 bc	4684.0 bc	25.35 c	23.00 c
H ₈	7.00 ab	16.33 a-c	9.33 ab	42.00 bc	42.10 a-c	1258.7 b	4859.7 b	25.89 bc	24.20 b
LSD at 5%	0.7761	1.7564	1.216	1.6602	4.3841	123.00	191.85	1.9018	0.8747

H₁=Control (untreated), H₂=Hydro priming, H₃= Seed priming with 2% Humic Acid solution, H₄= Seed priming with 4% Humic Acid solution, H₅= Soil application @ 2kg ha⁻¹Humic Acid, H₆= Soil application @ 4kg ha⁻¹Humic Acid, H₇= Foliar spray with 0.1% Humic Acid solution, H₈= Foliar spray with 0.2% Humic Acid solution.

Harvest index (%)

Data showed in the table: 1 indicated that statistically maximum harvest index (28.64 %) was found where the humic acid was applied @ 4 kg ha⁻¹ in treatment (H6) however; it was at par with the treatment where the soil exposed @ 2 kg/ha humic acid in (H5) treatment. The least harvest index (19.05) was counted in control (H1). Maximum harvest index was accumulated with utilization of humic acid 4 kg ha⁻¹ that significantly affected plant metabolic process and also play a role in enzyme activation and plant nutrients which regulate the plant growth and development. Humic acid positively affect the cell membrane which leads to increase the transport of minerals, promoted photosynthesis, solubility of micro and macro elements, plant hormone-like activity, increased microbial populations and declined the active level of toxic minerals [65]. The results of current study are correlated with [66].

Seed protein content (%):

Data showed in table: 1 regarding seed protein content % statistically maximum seed protein content (25.20 %) was recorded where humic acid @ 4 kg/ha was added in soil (H6) however; it was at par with the

treatment where the soil application @ 2 kg/ha humic acid was done (H5). The lowest seed protein content (21.00) was obtained in the treatment where no humic acid was applied (H1). Humic acid application has positively affected the proteins, starch and total soluble sugars. [67] concluded that humic substances improved NO₃⁻ concentration in plants. Its application improved the enzymes activity like glutamate synthase (GOGAT) and glutamine synthetase (GS), which act in the accessibility of NH₄⁺, could increase the nitrogen containing organic compounds in plant [68]. Mahmoud [69] stated that the humic acid application had beneficially increased the yield, protein and oil contents of peanut. The results of current research are line with [70].

Benefit Cost Ratio

The data given in Table: 2 indicated that maximum benefit cost ratio (2.17) was recorded with 4 kg/ha soil application of humic acid (H6). However, minimum benefit cost ratio (1.11) was recorded from the control (H1).

Table. 2: Effect of humic acid application methods on benefit cost ratio of Mungbean

Treatments	Uniform cost/ha (Rs.)	Humic Acid Cost/ha with labor (Rs.)	Total cost of production/ha (Rs.)	Seed yield kg/ha	Gross Income/ha (Rs.)	BCR	Net Income/ha (Rs.)
H ₁	56177.00	00.00	56177.00	761.00	62402.00	1.11	6225.00
H ₂	56177.00	458.00	56635.00	891.70	73119.40	1.30	16942.40
H ₃	56177.00	608.00	56785.00	1009.70	82795.40	1.47	26618.40
H ₄	56177.00	758.00	56935.00	1105.00	90610.00	1.61	34433.00
H ₅	56177.00	858.00	57035.00	1396.70	114529.40	2.03	58352.40
H ₆	56177.00	1258.00	57435.00	1487.70	121991.40	2.17	65814.40
H ₇	56177.00	498.00	56675.00	1187.30	97358.61	1.73	41181.61
H ₈	56177.00	538.00	56715.00	1258.71	103213.40	1.83	47036.40

BCR=Benefit cost ratio

CONCLUSION

The findings underscore the potential of humic acid as a cost-effective and environmentally friendly growth enhancer for Mungbean cultivation, particularly in sustainable agricultural practices. Across all application methods, there was a positive impact on mungbean yield and quality. Specifically, soil application at a rate of 4 kg ha⁻¹ of humic acid resulted in the highest crop yield and protein contents. The consistent positive effects of humic acid across various application methods highlight its promising role in enhancing the overall productivity and quality of mungbean crops. In future, research can be focused on humic acid characterization and its narrower level for mung bean production. A research focusing on different sources of humic acid in varying soil types can also be conducted.

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